Molecular mechanisms supporting long-term recall

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The classical picture of memory storage in the brain, widely accepted at this time, asserts in essence that all memory is carried by the synapses in the form of synaptic strengths. However, synapses are known to be impermanent; there is significant synaptic turnover in the brain. Also, successive events of long-term potentiation (LTP) and long-term depression (LTD) overwrite synaptic patterns as time goes on. A separate and distinct issue is that all efforts to explain the large memory capacity of the human brain in terms of synapses alone have so far failed. The present paper outlines a model describing synapse-based memory as working in tandem with molecular mechanisms to support the storage and retrieval of memories. It is found that the memory-supporting molecules need not contain the detailed description of mental entities, as had been envisioned in the "memory molecule papers" from fifty years ago, but only the unique identifiers of the entities; and that this can be achieved using relatively small molecules, using a random code. It is argued that the molecules contain their information in the form of sequences of nucleic acids rather than amino acids. They must be able to "wake up" large sets of synapses together, as units, in order to achieve recall; and the implementation of such an operation requires on-the-spot creation of precise and specific receptors for the molecules. This is not possible with polypeptides, but it is possible with DNA and RNA sequences. It is noted that LTP/LTD, through the interplay between AMPA receptors and NMDA receptors, includes means for separating the potentially meaningful signals from the random synaptic bombardment (by responding to surprising multi-neuronal events). The memory model described is closely linked the cell assembly hypothesis; its method of bringing back distant memories works through reconstructing their cell assemblies.

CHAPTER 1. INTRODUCTION

The discovery of long-term potentiation (LTP) and long-term depression (LTD) (Lømo, 1966; Pinsker *et al*, 1970; Bliss and Lømo, 1973; Bliss and Gardner-Medwin, 1973; Dudek and Bear, 1992; for reviews see Bliss and Collingridge, 1993; Malenka and Bear, 2004; Collingridge *et al*, 2010; Kandel *et al*, 2014) inspired a powerful surge in published research during the past decades, through its promise to open up the molecular biology of memory storage to detailed experimental study.

The mechanistic linkage of memory, as the concept is known from psychology, to LTP and LTD is not clear-cut; and attempts to make strong statements on the subject have been criticized (Malenka and Bear, 2004). But the relevance of the data to memory is clear; it is inferred from the fact that LTP and LTD both cause changes in synaptic efficacy (lasting some weeks or months); and it is generally accepted that the storage of memory is connected to synaptic change.

The exact nature of linkage between memory and the synapses is, once again, not clear-cut. The "classical" view of memory, tacitly accepted by many workers in the field, goes further than the mere statement of linkage; it *equates* memory to the pattern of synaptic weights. It states, broadly speaking, that memory is governed by the gradual evolution of synaptic strengths, under Hebbian (1949) and other rules; and that the behavior of the brain is determined by whatever happens to be their latest configuration. In essence, this view of memory states that the memory trace is made up of the synaptic strengths and nothing else (see Martin *et al*, 2000 for a summary of the arguments). The contributions of synaptic scaling and structural plasticity have in the recent years been carefully evaluated under the classical rules, and careful capacity estimates have been added (Tetzlaff *et al*, 2013; Knoblauch *et al*, 2010, 2014; Fauth *et al*, 2015).

However, the classical view which anchors all learned information in synapses is clearly an oversimplification. One problem with taking it literally is that the synapses are not permanent; there is significant synaptic turnover in the brain (Lendvai *et al*, 2000; Trachtenberg *et al*, 2002; Stettler *et al*, 2006).

Even when the synapses themselves survive for a long time, successive events of LTP and LTD will tend to counteract and overwrite each other as time goes on (Abbott and Nelson, 2000; Sjöström et al, 2001), obliterating old patterns of synaptic weights and covering them with layers of new ones. Once again, we are led to the conclusion that the pattern of synaptic strengths cannot be relied upon to preserve, for instance, childhood memories.

The present paper contains arguments to expand the functional linkage between LTP/LTD and memory storage, and to modify the dogmatic "classical" approach. The scheme of memory proposed preserves the synapse-centered view but supplements it with an

intracellularly based mechanism of bringing back sets of synaptic weights, together as units, to wake up old memories when suggested by contextual cues. This requires what may be called "event-selective tagging" of synapses, meaning that when synapses are enhanced as a part of late LTP, they must receive, in a manner to be discussed, unique identifiers of the multisynaptic events which originally qualified them for being selected.

CHAPTER 2. MOLECULAR REQUIREMENTS OF LONG-TERM MEMORY

2.1. Critique of the "classical" view of memory

Synapses do not last forever. New synapses can arise in an adult brain, while other synapses disappear in comparable numbers (Lendvai *et al*, 2000; Trachtenberg *et al*, 2002; Stettler *et al*, 2006). A quantitative value has been attached to synaptic turnover by Stettler *et al* (2006), who examined the appearance and disappearance of axonal boutons in the intact visual cortex in monkeys, in a context where no experimentally induced learning or trauma could be held responsible, and found the turnover rate to be 7% per week – which would give the average synapse a lifetime of a little over 3 months.

Dramatic changes in overall synaptic spine counts have also been found during certain changes of the global environment. In an electron microscope study, Kirov *et al* (1999) compared spine counts between perfusion-fixed hippocampal material and slices in which the neurons were kept alive but were deprived of their usual input. They found that the slices contained 40-50% more synaptic spines, and determined that the added spines appeared in the first few hours after slicing. The extra spines were verified, through serial-section electron microscopy, to carry full-fledged synapses with all the usual presynaptic and postsynaptic elements.

Spine counts can also undergo dramatic change without laboratory intervention. Female rats in the 24 hours between proestrus and estrus show a 30% decrease in the spine counts in CA1 pyramidal cells; then the spine counts return to their earlier levels during the rest of the cycle (Woolley *et al*, 1990).

It has, further, been found that under stress axons in the cortex can sprout new branches and let old ones disappear in substantial numbers, taking all their synapses with them (Yamahachi *et al*, 2009), indicating that even the configuration of synapse-carrying fibers in the brain is not stable.

The issue has been addressed in a general form, in terms of synaptic competition schemes (Changeux and Danchin, 1976), whereby newly formed synapses, formed only where needed, can displace some of the older ones. It is implied that the well-placed location of newly formed synapses allows us to overlook the massive disappearance of formerly installed synapses. The implicit assumption is that the brain has so many synapses that if they are judiciously allocated the combinatorial diversity of synaptic patterns will easily support all memories of a lifetime.

The problem with this assumption is that in the higher animals neurons must act in groups when exerting their effect on the network, and accordingly the number of independently adjustable synaptic weights is limited by the group structure. The maximum number of possible groups and subgroups that can fit into a network under any given allocation of synaptic weights depends on the specifics of modeling. But, if the requirements of reliability and retrievability are respected, the number can only be increased to a certain point. In an early set of calculations (Legéndy, 1967; Scott, 1975,1977; Hebb, 1976), an attempt was made to estimate the largest possible number of groups, allowing the parameters unknown at the time to assume whatever values would maximize the capacity and considering the network to be randomly connected (roughly approximating CA3 in the hippocampus). The number seemed almost acceptable at the time (about 10^9 coordinated neuron groups); however, more recent data force us to reduce it by several orders of magnitude.

The problem is still open; and to my knowledge no purely synapse-based model is able to account for the large capacity of the human brain. Hence the attempt, in the rest of this paper, to update the "classical" model of memory.

2.2. LTP and LTD – micro-statistical computations by biological analog means

The data on plastic change have one subtle aspect with far-reaching consequences: The multisynaptic input combinations found to induce LTP and LTD closely match the definition of "surprising events" (Legéndy, 1970, 1975, 2009; Palm, 1981a, 2012). *Surprise*, in the neuronal context, is defined as the negative logarithm of the probability that a pattern of firing occurs under the baseline spike statistics (for instance Poisson distribution in the case of *Poisson surprise*; Legéndy and Salcman, 1985). Said differently, it is defined as the degree to which a pattern is unlikely to occur by accident.

The mechanism whereby the interplay between AMPA receptors (AMPARs) and NMDA receptors (NMDARs) results in sensitivity to surprising events is illustrated in Fig. 1.

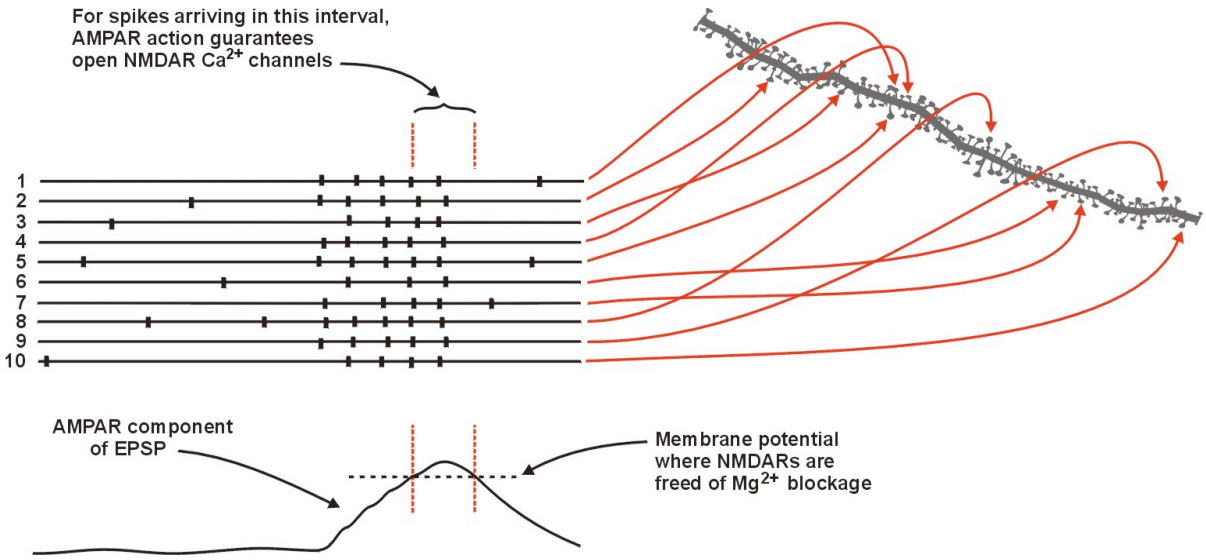

Fig. 1. The role of AMPAR-NMDAR interplay in surprise detection.

Hand drawing of a number of spike trains (left), incident on synapses (arrows) on a segment of a dendrite (right). The drawing includes a brief episode where volleys of nearly simultaneous spikes occur on the channels shown, a few times during a brief period of time -- a compound input event comparable with ones found to induce a dendritic spike and LTP (Remy and Spruston, 2007). As seen, by comparison with the background firing, the repeated coincidence is highly improbable under the baseline spike statistics (is highly surprising). The spike trains arriving to the other synapses on the dendrite segment (not shown) remain at their baseline spike rates. The EPSP component due to the AMPARs alone is shown at the bottom in rough sketch; and the level of membrane potential at which the Mg^{2+} blockage of the NMDARs is removed is shown as a horizontal broken line. It is seen that all the synapses receive spikes in the time interval where the Mg^{2+} blockage is removed and the Ca^{2+} channels are open.

It is noted that the membrane potential wave shown in Fig. 1 is not the EPSP but only one component of the EPSP; it excludes the contributions of the Ca^{2+} ions passed through the NMDA receptors (and the contributions due to voltage-gated calcium channels, as well as the secondary changes from Ca^{2+}-initiated signal transduction pathways), even through the depolarization from the massive calcium (and sodium) influx accompanying the subsequent dendritic spike often overwhelms the original linear potential (Golding *et al*, 2002; Remy and Spruston, 2007). This is intended to emphasize that (to a good approximation) when the AMPAR component of depolarization is insufficient for reaching the Mg^{2+} threshold, the Ca^{2+} ions do not get a chance to make their contribution; and there can be no long-term plastic change.

The AMPAR-NMDAR interaction, together with the Mg^{2+} threshold requirement, enforces a combination of circumstances which together ensure low probability of accidental occurrence. First, the arrangement enforces multisynaptic coincidence (*cooperativity*) of firing. Second, the contributions from miniature EPSPs will only be additive if the time spread of their coincident stimulation is narrower than the individual decay times. Third, the cooperative stimulation must repeat enough times before decay of the EPSPs to rise above the Mg^{2+} threshold. All this must occur in order for the NMDARs to make their contribution (which ensures further repetition). The calculation of surprise (omitted here in favor of the graphical demonstration) utilizes the extremely low probability of the joint event where sufficiently many spikes arrive to sufficiently many synapses in a sufficiently narrow time interval to have their desired effect.

The concept that surprising events should play a crucial role in inducing plastic change has been proposed on theoretical grounds a number of years ago (Legéndy, 1970, 1975); but at the time there was no evidence that they actually occurred in the brain. Since then evidence has become plentiful, partly through the prominence of LTP/LTD in all brain regions (Malenka and Bear, 2004), partly through the frequent occurrence of dendritic spikes (Svoboda *et al*, 1997; Kamondi *et al*, 1998; Losonczy and Magee, 2006; Spruston, 2008; Major *et al*, 2013; Smith *et al*, 2013; Palmer *et al*, 2014), which also require

surprising input; and, independently, through the wealth of high-surprise single-neuron events seen in the awake cortex (Legéndy and Salcman, 1985).

There is an appearance of contradiction between the low probability of some surprising events, so low that they should essentially never occur, and the observation that in fact they are quite frequent in the brain. The resolution of the paradox is that surprising events are only improbable when viewed in isolation; when they are viewed as the local manifestations of area-wide cooperative events, as predicted by the cell assembly hypothesis (Hebb, 1949), their occurrence becomes natural.

The overall functional concept emerging is that the brain makes use of prominent network-wide events (the *ignitions* of cell assemblies -- Rapoport, 1952; Legéndy, 1967; Palm, 1981b, 1982; Wickelgren, 1999; Legéndy, 2009), to reach the neuronal localities; then, having reached them, it employs micro-statistical analog means (LTP and LTD) to register them as specific patterns of modified synapses.

The functional justification of the arrangement is that the brain is noisy. If the signal-carrying events were quietly inserted with the rest of the spike trains they would be drowned beneath the noise; but if they are broadcast through ignitions, which reach many localities in the form of surprising events, they become detectable with a high degree of reliability.

The *surprise*-based view of synaptic plasticity (Legéndy, 1975; von der Malsburg, 1995) is alternative to the *average-spike-rates*-based view (Shadlen and Movshon, 1999). Both describe plastic changes as being governed by the statistical properties of spike trains; the difference is that the surprise-based view is tailored to the demands of quick neuron-level decision making. Also, in the spike-rate-based view, the step of spike rate averaging throws away the brief episodes of correlated multineuronal firing, like the one illustrated in Fig. 1, and as by doing so throws away much of the signal with the noise.

2.3. Surprising events and their underlying causes. Graphical notation

By the usual syllogism of statistical inference, when a highly surprising firing event occurs, even once, its occurrence lends support to the assumption that the occurrence has reasons outside the mechanisms determining the baseline statistics; this in turn has the special advantage that the reason may be experimentally detectable. In statistics-based scientific research the equivalent of a surprising "event" can sometimes be an entire research project from beginning to end, and the surprise computation is based on the probability that the results of the study are all accidental. In the case of neuronal spike trains, the surprising event is generally brief; for instance brief enough to induce LTP. The underlying cause of the event cannot be known at the locality of the occurrences; however, the descriptive details of the surprising event, such as the set of synapses affected by its volleys, are capable of being recorded.

Importantly, different surprising events tend to modify different sets of synapses; and when they carry sufficient entropy (Shannon, 1948), their recorded synapse sets can contain a unique signature of the events and their underlying causes. The distinction between *surprise* and *entropy* gains prominence at the stage of recording. The surprise content indicates whether the pattern is distinct from the surrounding noise; the entropy content indicates whether the pattern is rich enough to be recognized later if its details are recorded. (For instance: prolonged tetanic stimulation of a single synapse is highly surprising – but it is entropy-poor.)

When a surprising event is sufficiently entropy-rich to be recognized later, it becomes meaningful to speak of "recurrences" of the event. It can then be said that when the event recurs at a later time, the recurrence is likely to signify the presence of *the same underlying cause* as it did earlier; and that if a neuron is equipped to *respond* to the recurrences, its responses (with high probability) signify the same *underlying cause* as they did at recording.

As mentioned, the underlying causes of surprising events are envisioned here as amounting to the underlying causes behind the ignitions of cell assemblies; and the recognition of ignitions amounts to recognition of the cell assemblies themselves. The present paper bypasses the question of how real-world items are made to correspond to cell assemblies; it takes the position that the rest of the brain can achieve that somehow. Its assumption of the paper is confined to the end result: it states that when a surprising event imparts enough entropy to be recognized later, its synaptic pattern generally corresponds to something worth recording.

This, then, is the rationale behind Fig. 2, and the assertion, implicit in the drawings that the indicated spike patterns signify describable objects. Here and in the figures below I deliberately use a graphical notation in which patterns are not designated by symbols like $a_1, a_2, \ldots, a_n$, but by icons representing visually clear-cut items, such as birds and leaves. It will be appreciated that the things which frequently underlie the surprising events are not easy to illustrate in drawings: these include sounds, locations, colors, actions, contexts of actions, or components of any of the above. What they all do have in common, though, is some element of physical reality which permits them, with luck, to be isolated experimentally.

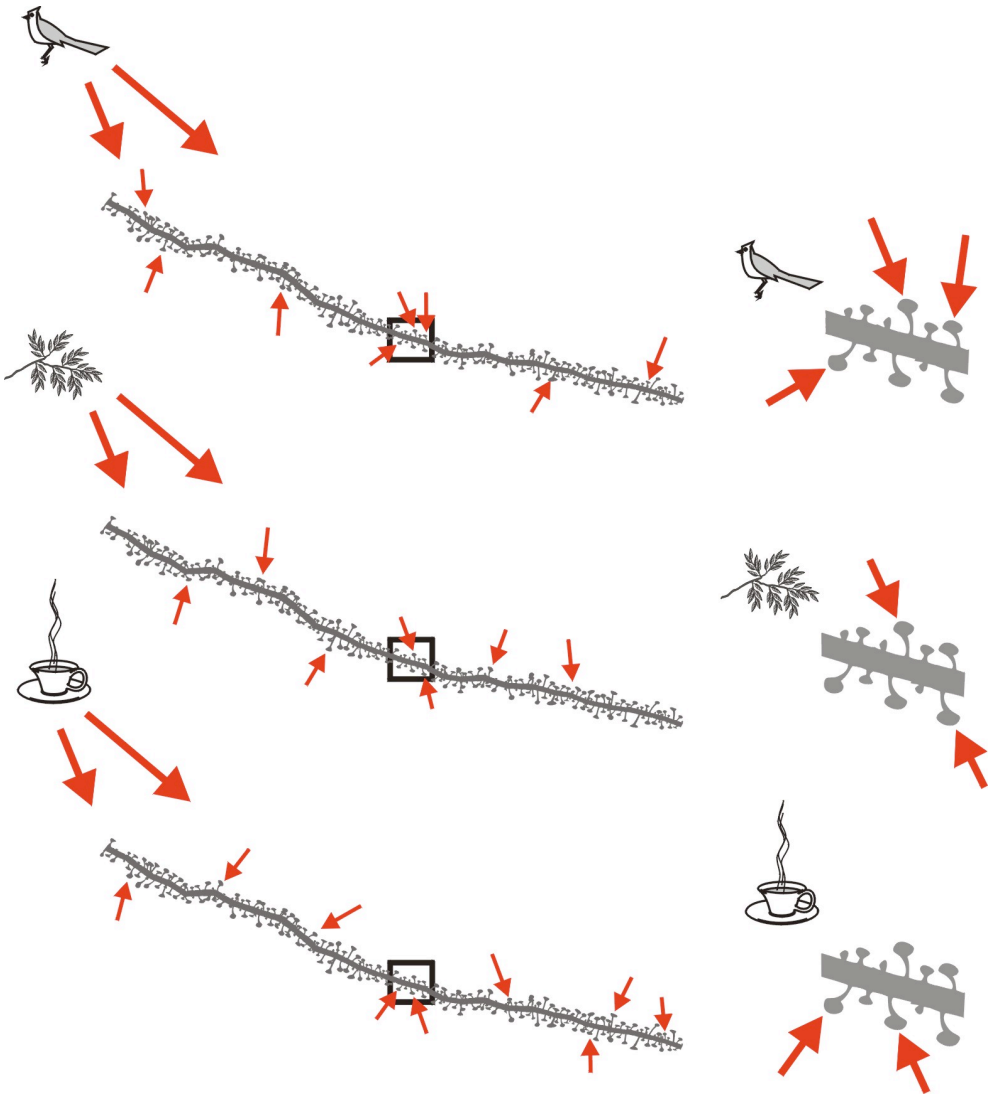

Fig. 2. Meaningful input patterns incident on a segment of dendrite.

Synaptic patterns of input volleys, arising from the ignitions of area-wide cell assemblies, can be designated by symbols representing the underlying causes of the volleys. A segment of a dendrite, sketched as having about 160 viable synapses on it, is shown in three copies, each receiving a volley of spikes (arrows) on a subset of its synapses from presynaptic elements (not shown). The icons of "bird," "leaves," and "coffee," stand for items represented by various cell assemblies, or, alternatively, for items communicated by the volleys of spikes. A short segment of the dendrite (framed) is magnified at the right of each drawing, and is reproduced in Figs. 4 and 5, in different contexts.

Among the conditions inducing LTP/LTD emphasis has here been placed on the role of presynaptic firing rather than postsynaptic (Hebbian) firing. This is meant to underline the role of surprise as a necessary condition in inducing both LTP and LTD (necessary but not sufficient).

The functional role of the postsynaptic spike is in fact not entirely clear at present (beyond a role in helping to intensify the depolarization). It is noted that intense presynaptic assaults can cause dendritic spikes (and often action potentials) without inducing LTP/LTD (Losonczy and Magee, 2006) and conversely, presynaptic assaults and dendritic spikes which do not cause action potential can nevertheless induce LTP (Remy and Spruston, 2007). Since spike timing can make the difference between strengthening and weakening the participating synapses (Abbott and Nelson, 2000; Sjöström et al, 2001), it is possible to argue that spike-timing-dependent plasticity (STDP) is merely a mechanism for achieving sharper synchrony within igniting neuron groups. (Because: when a

postsynaptic spike is delayed with respect to the bulk of the presynaptic assault, the strengthening of synapses reduces the delay; and when the postsynaptic spike leads with respect to the bulk of the assault, the weakening of synapses reduces the lead.) Accordingly, the intensity of presynaptic assaults will continue to be emphasized below as being the primary reason for plastic change.

2.4. The issue of unique labels: Shannon's random code ensemble

Mental entities within the brain are accessible at will; they are not constrained to any predetermined order in which they can be called up. In the implementation of memory retrieval this means that the calling agent must have a way to single out the mental entity to be retrieved, for instance through a unique identifier of some form.

Uniqueness of such identifiers is crucial to their function. Unique labels, such as serial numbers, are present in computerized databases, where their uniqueness can be guaranteed simply by making sure that no two items get the same label. The problem is that in the brain the same thing is not possible. In the brain new items, such as new mental entities and new components of mental entities, are added to the system from varied and mutually independent sources all the time; and since these sources often do not communicate with each other, it is not feasible to design protocols to guard against repetitions in the assignment of unique identifiers. This is where the idea of a *random code* offers a solution.

It is said that no two people have the same fingerprint; but in fact the uniqueness of fingerprints is only probabilistic; it is not rigorously arranged in a systematic way; the fingerprints are randomly generated during early development of the skin; and their random variation has so many degrees of freedom that there are many more distinguishable ridge patterns than there are people.

Calculating the probability of accidental coincidences between randomly generated patterns is easiest in a formalized paradigm where the patterns are simply random sequences of binary digits; for instance one may envision a set of n-bit binary numbers ("code words"), each of them generated by flipping a coin *n* times. The arrangement, in its general form, is known as "Shannon's random code ensemble" (Shannon, 1957; Mézard and Montanari, 2009).

One can estimate the probability of accidental coincidences between random binary code words by using the "2n rule," which states that, if an ensemble has N code words, each made up of n randomly generated bits, then by choosing n to be at least twice the minimum number of bits needed to create N distinct words, the probability of accidental coincidence can be kept below 0.5. Further, each additional binary digit doubles the safety factor. For instance, if an ensemble has 256 code words then (since $256=2^8$) one needs 16 random digits per code word to ensure a 0.5 probability that no two code words are equal; and if instead the strings have 26 bits, the probability that any two are equal becomes 1/1024.*

* The "*2n* rule" can be proven as follows:

Let N = number of code words; n = number of random digits in each code word; P = the probability that some two code words are the same. Then:

$$P = \frac{N(N-1)}{2} 2^{-n} \cong 2^{-n-1} N^2 = 2^{-n-1+2\log_2 N}$$

Then if, for instance, the requirement is that the P < ½, the latter result can be written as

$$2^{-n-1+2\log_2 N} < 2^{-1};$$

or, alternatively, as $n > 2 \log_2 N$, which is what the "2n rule" states. As seen from the previous forms, each added binary digit in the code words halves P, or doubles the safety factor.

Random code ensembles are often used in communication networks because their code words are easy to generate; and when sufficient redundancy is introduced they have powerful error correcting capability (Hamming, 1950).

The molecular context is especially well suited for achieving great variability through random sequencing of either the four nucleotides in RNA and DNA or the twenty amino acids in proteins. One known example of the great variability that can be achieved by random sequencing is offered by the antigen-binding regions of the immunoglobulins (Edelman, 1972), which are generated through variable cutting and pasting of gene segments (Market and Papavasiliou, 2003).

In the sections below, arguments will be presented that to overcome the limitations of "classical" synapse-based memory storage it is desirable for the brain to have a way to attach random-coded molecular labels to surprising events. As was seen, relatively small molecules can in this way encode a vast number of different labels.

In the present context, where the labels are needed in later retrieval of the information, it is necessary to generate, with each unique molecule, a matching molecule which can recognize it by key-to-lock matching. This is easier in nucleotide sequences than in peptide sequences, and accordingly the illustration in Fig. 3 implies that the labels use random segments of small non-coding RNA molecules.

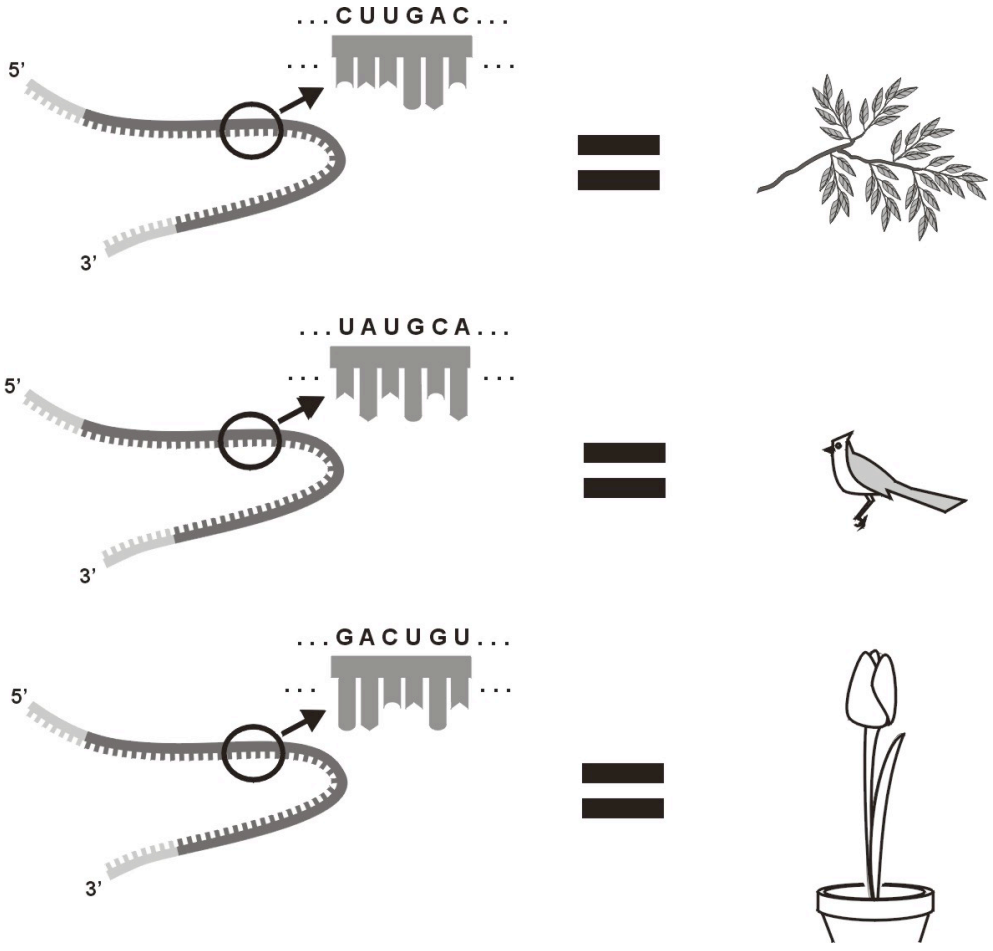

Fig. 3. The "event ID molecule."

<u>Different event ID molecules differ in the random sequence contained in their variable segment</u>. The variable segments (darker grey) contain random nucleotide sequences, with samples in the insets arbitrarily chosen for illustration. The icons on the right stand for the mental entities designated by the molecules.

In an earlier version of Fig. 3, the illustrative arbitrary sequences were shown as "… PHE-ASN-ALA-ASP …," "… PRO-ARG-GLU-SER …," "… ALA-LYS-THR-LYS …"; in other words the "code words" were assumed to be random sequences of amino acids, as in the immunoglobulins. But then I realized that the ID molecules are only useful (see below) if there is a quick way to create receptors for them specific enough to bind to them and only to them (noting that to meet the reliability requirements of the brain, the receptors must be less tolerant to mismatch than are many of the protein receptors.) The problem is that to construct a receptor to fit a randomly generated polypeptide, and do it fast enough for use in imprinting, would require a biological mechanism for quick construction of a peptide sequence whose conformation matches against the 3-D shape of a random polypeptide never seen before. And this is probably not possible. (If it were possible, our immune system could instantly defeat any infection.) However, in RNA and DNA sequences it is possible.

2.5. <u>The case for placement of event-selective tags at synapses</u>

The synaptic tagging concept (Krug *et al*, 1984; Frey and Morris, 1997; Sanes and Lichtman, 1999; Martin and Kosik, 2002; Sajikumar and Frey, 2004; Sajikumar *et al*, 2007; Reymann and Frey, 2007; Redondo and Morris, 2011) was originally introduced as a step in the LTP/LTD sequence, interposed between the events of synaptic stimulation and the synthesis of proteins implementing long-term synapse modification. It is made necessary by the fact that the stimulation is generally quite brief – its effects are long gone by the time the local protein synthesis has a chance to get underway. The extra step consists of placing a marker near the stimulated synapses to single them out and serve as guideposts for molecular products which are to arrive later.

The problem is that if all the marked synapses receive the same manner of tagging, the resulting synaptic change is subject to the classical problem that successive waves of LTP and LTD will eventually wash out all differences between synapses updated in different contexts; and as a result older synaptic patters will become irretrievable. The robust way in which actual brains can withstand the arrival of ever-newer waves of synaptic change tells us that the permanent synaptic changes at synapses are likely to be, in some way, event-specific.

This means that (in "synaptic tagging terminology") the molecules synthesized as part of the late LTP and deposited at the tagged synapses must themselves be tags. They must contain receptors able to recognize event ID molecules (Fig. 3) and "wake up" individual synapses in response to specific event IDs and not to others. The idea is illustrated in Fig. 4.

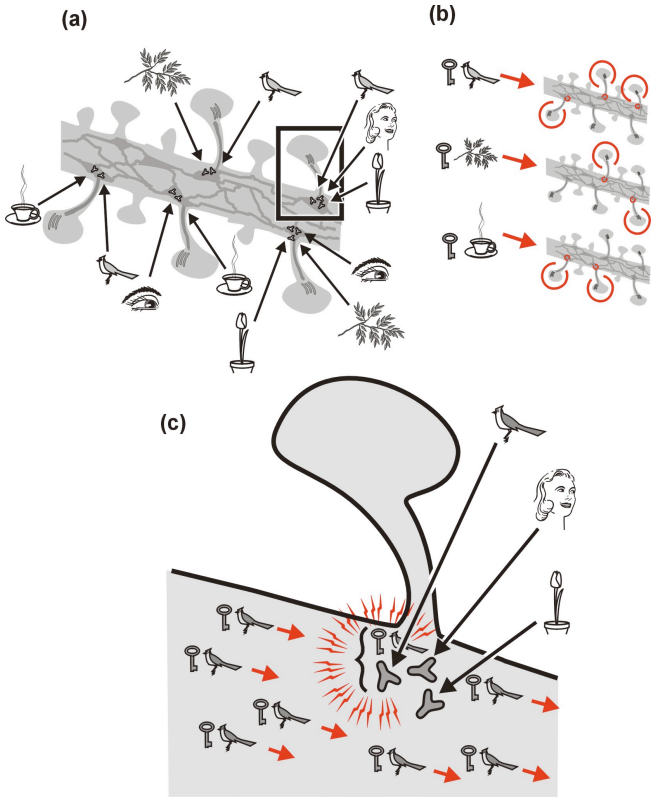

Fig. 4. Action of the "event ID molecule."

Waking up the synapses of an event by letting its event ID molecules single them out, via matching molecules pre-stored at the synapses. A piece of dendrite (reproduced from Fig. 2) is shown in rough sketch, including a number of "label" molecules, represented as Y-shaped outlines, which recognize the smaller messenger molecules (affixed with "keys"), like those in Fig. 3. (a) Event ID receptors for a number of entities. (b) The effect of three different swarms of event ID messengers, each waking up the synapses near its receptor (circled), in the same arrangement as in Fig. 2. (c) Same events as in the top drawing of (b), in magnified detail. A swarm of "bird" messengers moves past; one of them binds to its corresponding receptor (curly bracket) and initiates the synapse-waking action (sparks).

It is noted that, in the present context the range of items designated by "event ID molecules" includes a great deal more than simple objects; it covers anything and everything worth encoding in neuronal signals. For instance, the "event ID molecules" can carry the identities of situations continually present, such as smells or locations, as well as the identities of contexts in which actions may take place. Similarly, the multineuronal patterns giving rise to the identified surprising events can include sequences of ignitions and combinations of them.

Only under such a general interpretation of the *event ID* concept is it meaningful to say that "event ID molecules" can be tasked with "waking up" the synapses. It will be appreciated that one common reason for waking up a long-dormant set of synapses is that the *general context* of the action makes it likely that they will soon be needed; and in that case the arriving *event ID molecules* must be able to describe *contexts*.

2.6. Generating the event IDs: Outline of an event recognition complex (ERC)

The placement of event-selective markers at synapses, enabling event ID molecules to wake up the synapses, presupposes the existence of pre-built *sources*, able both to synthesize the event ID molecules and to do so at the right times. This, in turn, requires apparatus for recognizing the signals indicating what event ID molecules need to be sent out.
When discussing the apparatus for doing this, I will revert to the most rudimentary version of the "event ID" concept, where the events to be recognized and labeled are merely multisynaptic volleys from igniting cell assemblies; I will leave the more general version to future work.

In order to carry sufficient entropy, the recognition of multisynaptic volleys must be responsive to the identity of the synapses involved, rather than just the

number of active synapses (because only in that way can the recognition utilize the combinatorial diversity of synapse choices). This in turn implies the existence of a second kind of unique descriptor molecule, along the lines of Fig. 3 but with the difference that it encodes *neuron IDs* rather than multineuronal *event IDs*. The two ID molecules will be referred to as ID-1 and ID-2 molecules (for event ID and neuron ID, respectively). It is possible that recent experiments like those on neuron clones (Yu et al, 2009, 2012) will help point the way toward identifying the *neuron ID* molecule.

The required recognition apparatus, to be referred to as *event recognition complex* (ERC), needs to carry a number of receptors, one for each ID-2 molecule known to contribute to the multisynaptic event it is built to recognize, enough of them to meet the entropy requirement ensuring reasonable certainty for recognition. The outlines of the concept are sketched in Fig. 5.

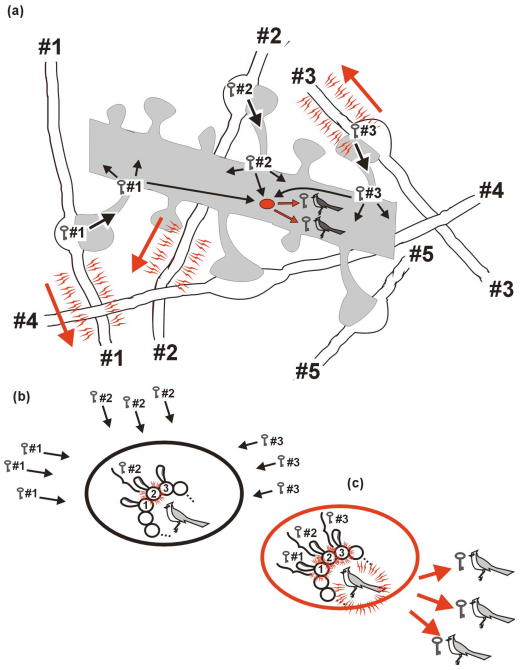

Fig. 5. Action of the event recognition complex.

An ERC, assembled to match the ID-2s sent out by synapses in response to an incoming volley, recognizes them as matching the "code words" pre-stored on its input receptors, then responds by producing a swarm of ID-1 molecules. (a) Neurons #1, #2, #3, bring firing (indicated by "sparks") and set off swarms of ID-2 molecules (denoted by their neuron designators with "keys" affixed to them) inside the dendrite. Some of these encounter a nearby ERC (ellipse) wired to respond to the "bird" combination. (b), (c) Recognition steps; the curved elongated symbols at the input terminals stand for the ID-2 receptors.

It may be assumed that ligand binding of the ERC receptors to ID-2s alters the conformation of their embedding proteins in a roughly additive way; so that when more different ID-2s bind to their receptors they cause greater deformation (in analogy to the wider opening of some ion channels when multiple agonists simultaneously bind to them). In this way it can be achieved that when enough ID-2s bind to the ERC to meet its entropy requirement, the machinery for synthesizing ID-1s is turned on.

Because the neuronal network is noisy, it is necessary to avoid, in some way, a situation where every presynaptic spike arriving in the course of random bombardment can initiate ID-2 release, and swamp the ERC with noise. Accordingly, it is expected that the intracellular release of ID-2s is triggered as part of the signal transduction pathways initiated by the Ca^{2+} ions from NMDA receptors. This would increase the likelihood that release only occurs at times of LTP/LTD, and only at the affected synapses; and enforce what has been described, in formalistic terms, as the noise-rejecting *surprise requirement* of plastic change.

Assembly of new ERCs (and the updating of old ones) is also expected to be part of LTP/LTD and, accordingly, also expected to be initiated through the NMDAR/Ca^{2+} signal transduction pathways. The platforms on which they are assembled are expected to be pre-built, probably on the endoplasmic reticulum (ER), so as to expedite the assembly by having all their pieces present to start with, except for the RNA sequences to be installed at the input end and the output end.

When the required calcium signals arrive, the platforms must first turn on a set of receptors for features shared by all the incoming ID-2s, then if receiving enough of them use the individual ID-2s as templates for building input receptors. Meanwhile, the nascent ERCs must also recruit a recently generated ID-1 sequence (possibly generated in an adjoining astrocyte), to serve as template to their output-synthesizing machinery. When the entropy of the recruited ID-2s is sufficient, the result will be a device able to recognize a multisynaptic input combination with good reliability and respond by issuing a unique ID-1 identifier.

It is expected that one of the extensions of the rudimentary ERC design of the last paragraphs is to expand the input machinery to include receptors for ID-1s, in other words not to confine the input molecules to *synapse identifiers* but extend them to *event identifiers*. The resulting stream of "indirect input" greatly increases entropy brought to the device; it also permits the "definitions" implemented by ERCs to be *recursive* – a potentially powerful concept.

It is likely that not all ERCs are located inside dendrites but some are inside the surrounding astrocytes. If the ID-2s produced in the dendrites (where the NMDAR/Ca^{2+} signal transduction pathways can reach them) can make their way into the astrocytes, the ERCs, by relocating in this way, come within easy drifting range of many more synapses than if confined to dendrites, and accordingly gain access to more entropy.

CHAPTER 3. DISCUSSION

It will be noted that the foregoing description emphasizes the decision-making role of *dendrite segments*; it does not treat *neurons* as the smallest indivisible functional units. A segment of dendrite can initiate dendritic spikes which often proceed to the cell body and generate action potentials; further, a dendrite segment can induce LTP (Remy and Spruston, 2007), and therefore initiate synaptic change. In general, in the context of interest here, a segment of a dendrite can act as a more-or-less autonomous decision-making and memory-storing element (Branco and Häusser, 2010; Major *et al*, 2013); it offers an attractive platform on which to discuss the microstructure of learning and memory.

The molecular contributions to memory, in the model outlined here, are closely linked the cell assembly hypothesis. The model restores memories by restoring their cell assembly (through waking up its supporting synapses); and it recognizes an item by recognizing its cell assembly (through identifying the synapses the assembly makes in the vicinity of an ERC). Both operations require that the cell assemblies are large enough to be recognized by limited subsets of their synapses.

The evidence supporting the large size of cell assemblies comes from the data on *dendritic spikes* and from *cross-correlation* studies.

Dendritic spikes are a frequent phenomenon in pyramidal cells and elsewhere; they have been seen both in slices and *in vivo* (Svoboda *et al*, 1997; Kamondi *et al*, 1998; Losonczy and Magee, 2006; Spruston, 2008; Major *et al*, 2013; Smith *et al*, 2013; Palmer *et al*, 2014). This is of interest in the present context because each time a dendritic spike is generated the conditions for its generation must, by necessity, be satisfied. The conditions include coordinated assaults of spikes on a segment of dendrite, which in turn require coordinated firing by large groups of neurons; because the rate at which random fluctuations would give rise to the needed assaults is far below the observed occurrence rate of dendritic spikes. Accordingly, dendritic spikes provide powerful evidence of large-scale coordination of neuronal firing.

The quantitative conditions of dendritic spike generation have been established by recently available glutamate uncaging data, which tell us that to initiate a dendritic spike requires time-concentrated volleys arriving to as many as a few per cent of all the synapses on the spike-initiating dendrite segments; for instance to about 20 synapses on the smaller dendrites of pyramidal cells (Losonczy and Magee, 2006; Polsky *et al*, 2009; Major *et al*, 2013). It is clear that the igniting cell groups behind the assaults must be large enough that such volley intensities are not far above the expectation value. This in turn also makes them large enough to be recognized on the basis of the samples reaching the dendrite segments.

Cross-correlation data lend further support to the large size of ignitable neuron groups. The probability of finding neuron pairs with strong positive cross-correlation is often quite high, especially when the pairs are selected to have similar response properties (Ts'o *et al*, 1986; Hampson *et al*, 1996) – and this is even true in neuron pools where only 1-2% of all neuron pairs are synaptically connected (Deuchars and Thomson, 1996; Hampson *et al*, 1996). Since in the latter cases direct excitation can create very few counts, the substantial numbers of extra counts

observed in the central bins of a cross-correlogram are overwhelmingly likely to come from coordinated firing by a group of cells (a cell assembly) in which both of the recorded neurons are members. If the cell assemblies were small, the probability of positive cross-correlation would be small.

The idea of molecular memory storage has been explored in the 1960s and 1970s; but has since then been abandoned. As reviewed by Morange (2006), some of the "memory molecule papers" have obtained valuable results but over-interpreted them (Hydén and Egyházi, 1962; Flexner *et al*, 1962; Ungar *et al*, 1972), while others have been fully discredited (McConnell, 1962; Babich *et al*, 1965). The effort, which had taken center stage at the time, has by now fallen into disrepute. The present paper attempts to revive the idea of molecular storage by pointing out an entirely new direction in which to seek evidence of the concept. The molecules do not describe the physical details of mental entities, as implied in the old papers; they only provide unique identifiers for them, which are not transferrable between organisms because they are randomly generated.

A note is in order on the subject of restoring old synaptic patterns, to account for the possibility that synapses often disappear after a while, and after they do the anatomical substrate required by Fig. 4 is no longer available. In this case the recreation of synapses involves growth, and that requires locally available information as to the target of the growth. The identity of the formerly presynaptic neurons must be preserved inside the dendrite as part of the long-term synaptic information, in the form of an ID-2 molecule paired up with to each ID-1 molecule. When the ID-1 part of such a pair recognizes the need for reviving its synapse, the attached ID-2 part has the information needed to seek out the right axonal process among the ones nearby (provided it is there).

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